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Microalgae Harvesting via Flocculation: Impact of PH, Algae Species and Biomass Concentration

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Abstract

Microalgae harvesting uses high energy input which makes large scale commercial cultivation economically unfeasible. Flocculation is the one of the most promising cost and energy efficient alternative method for harvesting microalgae biomass. In this work, pH based flocculation of three microalgal species *Chlorella vulgaris, Scenedesmus obliquus* and *Chlorococcum sp* were studied under varying pH levels and initial biomass concentrations. The biomass concentration was varied to each microalgal species tested ranging from 0.52 -2.11 g/L for *C. vulgaris*, 0.63 - 2.55 g/L for *S. obliquus* and 0.47 - 0.91 g/L for *Chlorococcum sp*. Impact of pH on flocculation was studied by adjusting the pH from 2 to 12 using 1 N HCl and M NaOH. The surface charge of the algal cells studied in this work was negative and varied between -12.2 to -17.4 mV. The flocculation efficiency based on pH values varied from one species to another. For *C. vulgaris*, pH of 3.5 and 9.5 resulted in highest flocculation, whereas pH of 4.0 and 9.0 had produced maximum flocculation in *S. obliquus*. The cells of *Chlorococcum sp* flocculated at higher levels at pH 3.5 and 9.0. The results indicated that flocculation efficiency is pH based and is dependent on microalgae species and initial biomass concentration.

Keywords

Flocculation; Biomass; Microalgae harvesting; *Chlorella; Scenedesmus; Chlorococcum*

Introduction

Microalgae are currently considered to be the most promising new source of biomass. Microalgae can produce more biomass per unit land area than agricultural crops depending on the technology used and the local climate. However, for microalgal biomass to become a commodity like most agricultural crops, the cost of production has to be reduced. The past years have seen an explosion in research and development on microalgal biomass production. Much progress has been made in increasing the yield through photo bioreactor design, selection of strains and genetic engineering of metabolic pathways. Much less progress has been made on research and innovation in downstream processing, although this is essential to reduce the cost of the production process. Today, microalgal production is rapidly moving from lab- and pilot scale to full-scale installations, prompting the need for cost- and energy efficient downstream processing technologies.

Cultivation of microalgae for biomass, lipid and other value added products involves separation of biomass from the growth medium. Harvesting process involves separation of microalgae from the growth medium however due to their small size, negative surface charge and similar densities to that of water makes the harvesting process as a difficult task [1-2]. During harvesting process, a major challenge lies in separating the microalgae from their growth medium. Several methods such as centrifugation, filtration, flotation and flocculation are being used for the harvesting of algal biomass [3-4]. Centrifugation can efficiently harvest most algal species but the capital and operational costs are high. Filtration is suitable for large algal cells but fouling issues associated with this method restricts it use. Floatation is another effective method of harvesting microalgae but is species specific and costly. The above methods require high energy inputs for harvesting of the algal cells which makes large scale production of microalgae for target products as not economically viable [5]. Flocculation or sedimentation is a most promising technique due to its low cost and energy requirements. During flocculation, destabilized particles are induced to coagulate, make contact, and thereby form larger agglomerates with higher sedimentation rate [6].

Metal coagulants are used for harvesting microalgae but their use results in high concentrations of metals in the harvested biomass. These metals remain in the biomass residue after extraction of lipids or carotenoids [7-10]. Chemical methods of flocculation using inorganic and organic flocculants are in practice but have contributed to high cost and maintenance [11]. Bioflocculation involves flocculation induced by polysaccharides

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and proteins derived from microorganisms [12-14]. The aim of the present study was to investigate and evaluate flocculation based harvesting processes as primary concentration step for microalgae biomass production. This research will focus on defining the variables such as pH, algal species and biomass concentration that influence flocculation process.

Methodology

Microalgal Strains and Culture Conditions

Chlorella vulgaris, Scenedesmus obliquus and *Cholorococcum sp* isolated as described earlier were used in this study [15]. The microalgae were grown in Bristol's medium in a 250 mL conical flasks under controlled conditions (20±1°C Temperature; 31±1% relative humidity; 140 µmol photons/m2/s; 12:12 h light/dark cycle,). Cultures were aerated for 8 hours daily with atmospheric air (0.05 L.s-1).

Flocculation by pH Change

The effect of medium pH on flocculation efficiency was carried in test tubes with 20 mL of medium. Based on the initial pH (6.5-7.5) of the growth medium, pH value of each sample was gradually adjusted by addition of 1N HCl (pH 2 to 6) and 1M NaOH (pH 8 to 12). The glass tube was vortexed thoroughly after the addition of acid/alkali for 30 seconds and allowed to stand at room temperature for 15 min. Then an aliquot of medium at the height of 2/3rd from the bottom was pipetted for measuring the optical density at a wavelength of 750 nm (OD_{750}).

Determination of Flocculation Efficiency

The flocculation efficiency of each sample will be calculated according to following equation:

Flocculation efficiency % = (1-A/B) x 100

Where,

A is the OD_{750} of sample

B is the OD_{750} of culture medium.

Zeta Potential Determination

The zeta potential of three microalgal algae species was measured by using a Malvern Nano Zeta Sizer ZS90 (Malvern Instruments Ltd.) using 1 mL cell dispersions in their respective medium within a pH range of 2 to 12. Zeta potential was performed in triplicates at room temperature and the average values were considered for data.

Determination of Dry Weight

Algae biomass concentrations were calculated by determining the dry weight of microalgae. Algal suspension was filtered through a pre-weighed Whatman filter paper (0.45 µm) and the filter paper was dried in an oven at 105°C for 48 hrs. Dry weight was calculated by subtracting the final weight from initial weight and expressed as biomass concentration (g/L).

Statistical Analysis

All the flocculation treatments were run in duplicates and the means of the efficiencies were analysed using one-way ANOVA. The significance level was established at P = 0.05 unless otherwise noted.

Results and Discussion

Large scale cultivation of microalgae requires high energy inputs and is not economically feasible as harvesting accounts for more than 30% of the total cost [16,17]. Flocculation is generally induced by addition of chemicals that interact with the negatively charged microalgal cell surface. These chemicals can induce flocculation through neutralizing the negative surface charge of the cells, connecting individual cells or forming a precipitate that binds the cells. In this study, effects of pH and biomass concentration on the efficiency of flocculation were evaluated. The biomass concentration was varied to each microalgal species tested ranging from 0.52 -2.11 g/L for C. vulgaris, 0.63 - 2.55 g/L for S. obliquus and 0.47 - 0.91 g/L for Chlorococcum sp. To test whether flocculation was reproducible, the flocculation experiments will be repeated on batch cultures grown with at least a one month interval.

The surface charge (zeta potential) of microalgal cells is most important characteristic which also affects flocculation nature of the biomass [18,19]. It also affects the dosage of an ionic flocculant required to produce effective flocculation. When the zeta potential is high (> 25 mV, positive or negative), electrical repulsion between particles is strong and when it is close to zero, particles can approach each other to a point where they will be attracted by Van der Waals forces. When that happens, particles will aggregate and flocculation or coagulation will occur. The surface charge of the algal cells studied in this work was negative and varied between -12.2 to -17.4 mV. At neutral and alkaline pH values, algal cells are known to have a net negative surface charge due to the ionization of functional groups [19-21].

Microalgae	Zeta potential (mV)
Chlorella vulgaris	-17.4 ± 1.2
Scenedesmus obliquus	-12.2 ± 1.7
Chlorococcum sp	-13.1 ± 0.8

pH based (acidic and alkaline) flocculation method was performed to three species of microalgae. The results imply that the effect of pH on harvesting microalgae will differ between species. As a result, pH range that has been tested for harvesting one species of microalgae may not necessarily be effective for another species of microalgae. This could be due to the variations of surface groups on the different strains of microalgal cells.

The cells of *C. vulgaris* began to coagulate at higher levels when the pH decreased from pH 6.0 to 3.0 and flocculation was observed when the pH further decreased to 2 but the coagulated cells remained suspended in the medium at this pH level (Figure 1 and 2). The flocculation efficiencies reached a maximum at pH 3.5

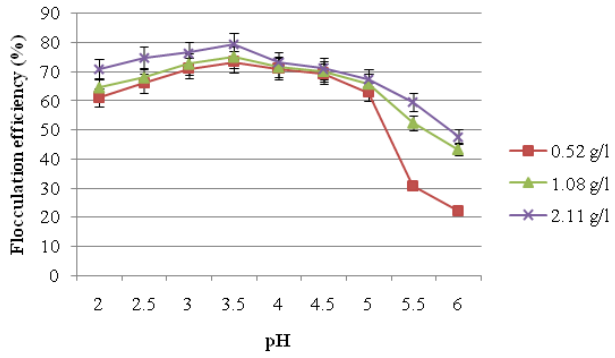

Figure 1: Flocculation tests of *Chlorella vulgaris* at acidic pH

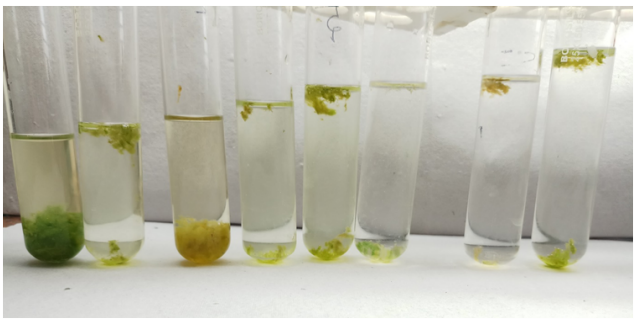

Figure 2: Flocculation efficiency of *Chlorella vulgaris* at acidic pH with 0.52 g/L initial biomass concentration

in all the biomass concentrations used with a slight decrease when the pH was lowered to 3.0, 2.5 and 2.0. In a study by Seo et al., harvesting efficiencies for *Chlorella* sp were higher when the pH was lowered [22]. Initial biomass concentrations of 0.52, 1.08 and 2.11 g/L of *C. vulgaris* were used to determine the influence of biomass concentration on pH dependent flocculation. For a given initial biomass concentration, an increasing amount of the biomass was removed as the pH was reduced to 3.5. An increasing concentration of the algal biomass in the medium increased the HCl dosage required for removal of a given percentage of the biomass from the medium. This must be due to the fact that more surface charge needed to be neutralized at higher biomass levels [23]. However, in some studies the harvesting efficiencies were higher when the pH was raised above 11 [24-26]. At lower pH values, the flocculation efficiency was higher even at higher biomass concentration. On the contrary, when the medium pH was increased, the flocculation efficiency was decreased to 74.6% and 65.8% with increasing biomass concentration of 1.08 and 2.11 g/L respectively (Figure 3 and 4). Another significant observation in the study is biomass settlement took place under 10 min at lower pH values (< 6) where as it took more than 25 min at higher pH values (> 8).

When the pH of S. obliquus culture was reduced to 4.0 by adding 0.1 M HCl, the flocculation efficiency was 70.2% at 0.63g L-1initial biomass concentration (Figure 5 and 6). Flocculation efficiency of S. obliquus was continued to decrease when the pH was reduced from 4.0 to 2.0 irrespective of the initial biomass concentration. This is due to the fact that the cells could have gained positive surface charge at lower pH which reduces the flocculation efficiency [9]. Higher pH values obtained by the addition of 1N NaOH resulted pH 9 as optimum for flocculation of *S. Obliquus* cells. Increasing the pH from 9 to 12 led to reduced flocculation efficiency. The highest flocculation (70.2%) was observed at pH 9.0 with 0.63 g/L biomass concentration (Figure 7 and 8). Besson and Guiraud noted that flocculation recovery efficiency was improved by the addition of NaOH [27]. Another finding of this study is flocculation efficiency was decreased with increasing initial biomass concentration of *S. obliquus*. There was a 18.6% reduction in the flocculation efficiency at pH 9.0 when the biomass concentration was increased from 0.63 to 2.55 g/L. Similar findings were reported by Wu et al., which flocculation efficiencies induced by pH increase was decreased considerably with increase of biomass concentrations [28].

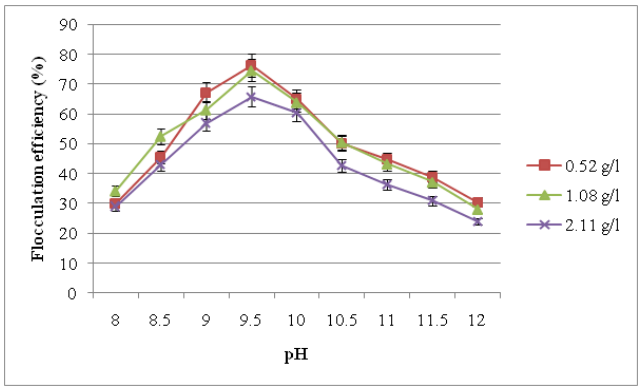

Figure 3: Flocculation tests of *Chlorella vulgaris* at alkaline pH

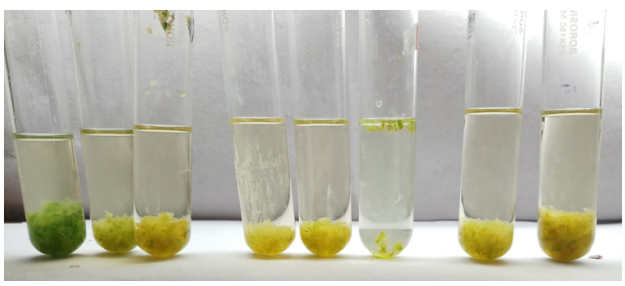

Figure 4: Flocculation efficiency of *Chlorella vulgaris* at alkaline pH with 0.52 g/L initial biomass concentration

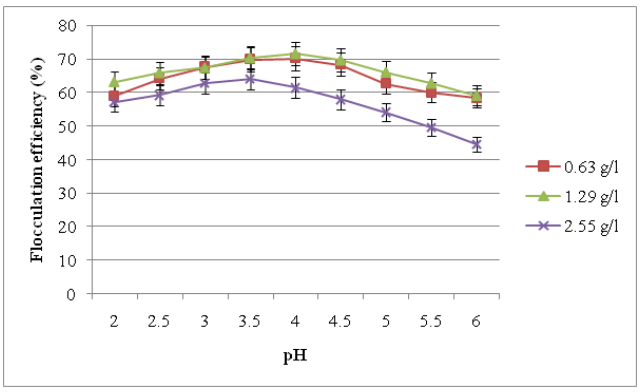

Figure 5: Flocculation tests of *Scenedesmus obliquus* at acidic pH

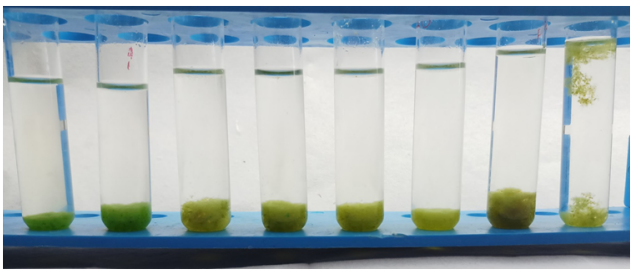

Figure 6: Flocculation efficiency of *Scenedesmus obliquus* at acidic pH with 0.63 g/L initial biomass concentration

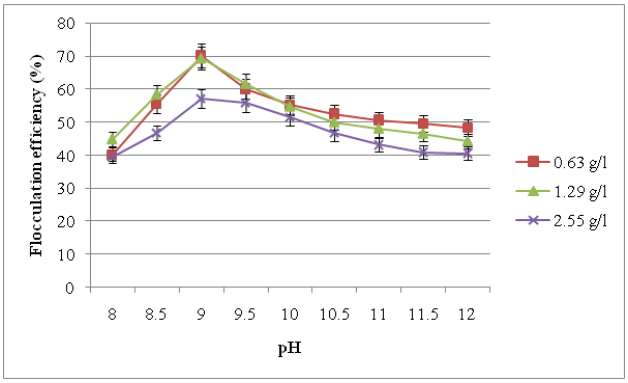

Figure 7: Flocculation tests of *Scenedesmus obliquus* at alkaline pH

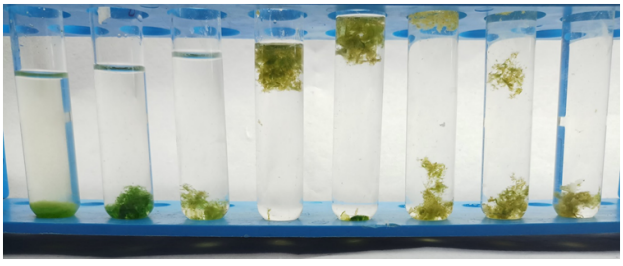

Figure 8: Flocculation efficiency of *Scenedesmus obliquus* at alkaline pH with 0.63 g/L initial biomass concentration

The flocculation efficiency based on pH values varied from one species to another. For example, pH of 3.5 and 9.5 resulted in highest flocculation in *C. vulgaris*, whereas pH of 4.0 and 9.0 had produced maximum flocculation in S. obliquus. The cells of *Chlorococcum* sp flocculated at higher levels at pH 3.5 and 9.0 (Figure 9 and 10). The differences in the chemical composition of the cell envelopes are a factor in affecting the flocculant dose requirement [6]. Different biomass concentrations of the *Chlorococcum sp* were used for flocculation experiments (0.47, 0.95 and 1.91 g/L). Flocculation efficiency in the range of 70.2 – 78.6 % was observed when the pH was between 2 and 4.5 at 0.47 g/L biomass concentration. For a given biomass concentration, an increasing amount of biomass was removed when the pH was reduced. Biomass concentrations influence the pH induced flocculation of microalgae and the efficiency decreased at higher biomass concentrations [28,29]. In this study, the flocculation efficiency was reduced when the biomass concentration was increased from 0.47 to 1.91 g/L.

At higher pH values, flocculation of cells was maximum at pH 8.5 and 9.0 but was decreased with pH above 9.5 and higher initial biomass concentration (Figure 11). By increasing pH of the solution, destabilization of cells and their precipitation occurs in the medium due to changes in isoelectric point of the solution. The use of flocculation induced by high pH for harvesting microalgae may have an additional advantage that the high pH may effectively sterilize the microalgal biomass. This was proven by the change of biomass colour from green to yellow with increasing concentration of NaOH (Figure 12). Another reason for the change of colour is due to the saponification of chlorophyll in the presence of sodium hydroxide [30].

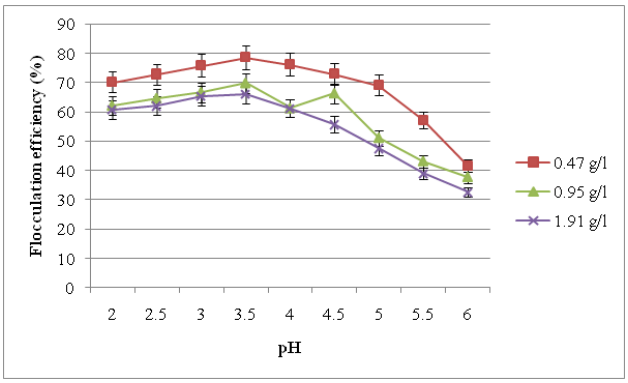

Figure 9: Flocculation tests of *Chlorococcum sp* at acidic pH

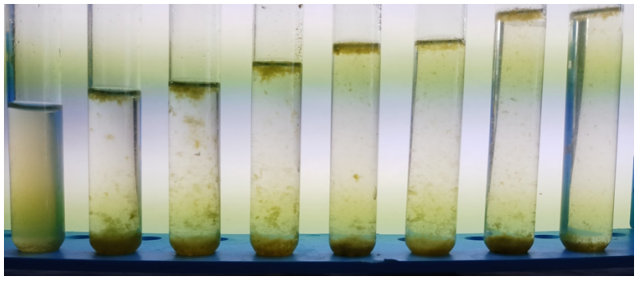

Figure 10: Flocculation efficiency of *Chlorococcum sp* at acidic pH with 0.47 g/L initial biomass concentration

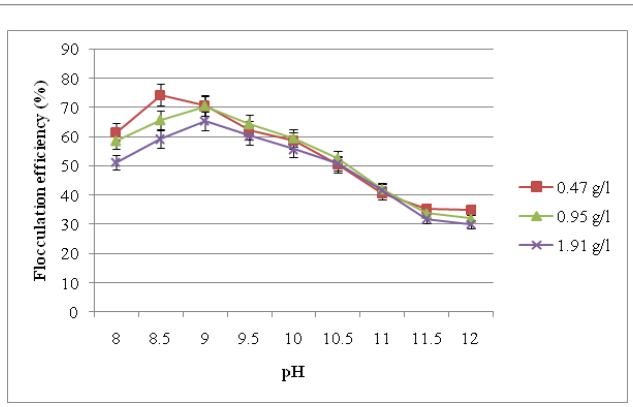

Figure 11: Flocculation tests of *Chlorococcum sp* at alkaline pH

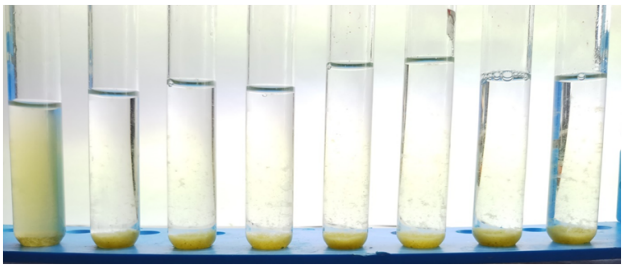

Figure 12: Flocculation efficiency of *Chlorococcum sp* at alkaline pH with 0.47 g/L initial biomass concentration

The results from this study showed that flocculation induced by pH change is a useful method to harvest microalgae. At acidic pH levels, the flocculation rates were higher in *S. obliquus* and *Chlorococcum* sp whereas alkaline pH produced greater flocculation of *C. vulgaris*. The results indicated that flocculation efficiency is pH based and is dependent on microalgae species and initial biomass concentration.

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